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Author: F. Gerstl, Bundesstaatliche Impfstoffgewinnungsantalt (National Serum Production Institute), Possingergasse 38, Wein XVI, Austria

Title: Contribution to the diagnosis and morphology of the virus producing contagious pustular dermatitis (Ecthyma contagiosum) in the chamois (Ein Beitrag zur Diagnostik und Morphologie des Erregers des ansteckenden Lippengrindes (Ecthyma contagiosum) der Gemse).

Journal: Journal for Bacteriology (Zentralblatt fur Bakteriologie) (orig.) vol. 195: 182-189 (1964).

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During the epidemic occurrence of the so-called papillomatosis of chamois during the winter of 1962-63 in Austria, experimental investigations were carried out by Grausbruber (9) which established the identity of this agent as contagious pustular dermatitis of sheep and goats. Within the scope of this work, it was of interest to examine under the electron microscope the virus of the so-called papillomatosis of chamois and to compare its morphology with that of the already identified infectious agent of contagious pustular dermatitis of sheep and goats.

Electron microscopic investigations of this agent in sheep and goats and Ecthyma contagiosum virus (E.C.V.) isolated from humans have already been carried out (1, 18, 19, 3). The E.C.V. is included in the Pox group (4, 3). It can be easily differentiated from the Vaccinia virus electron-microscopically (1, 18, 3). According to the literature, great similarities exist between the virus of stomatis papulosa bovis (24, 19, 3) and the virus of Milker's nodule (25, 20, 28, 14, 5) when they are examined electron-microscopically with regards to shape, size, structure, and axial ratios.

MATERIALS AND METHODS

Epidermal scales and papillomatotic lesions from expiredand isolated chamois, which had been sent to the National Institute for Contagious Animal Disease Control in Modling bein Wein (Director: Dr. F. Kress), were used as material for electron microscopic studies. In addition, fresh specimens were examined under the electron microscope after the transfer of infectious material through experimental animals (chamois, sheep, and goats). From the old epidermal scales and papillomatotic lesions, a suspension was prepared, which was studied for E.C.V. after high and low-speed centrifugation. From the artifically infected animals, the small, lentil-sized grey-white nodules were removed with a scapel on the fourth day of illness and were ground on a glass slide. Formerly, it had been found that it was advantageous to immerse the

the glass slide in a water bath for five minutes in order to wash away any water-soluble materials (colloids and blood constituents) until the slide is water-clear. Following shadowing with platinum-iridium, the slides were fixed in a 1-2 % buffered osmium tetroxide solution for 10 minutes. The secondary negative staining was carried out in a saturated solution of uranyl acetate with a pH of 4.2. In addition, several specimens were shadowed with gold-palladium in chloroform in a Sieman's steaming apparatus. As carrier films, 0.5 % mowital in chloroform and 0.5 % formvar in 1,2-dichloroethane (purest available) were employed. The photographs were made with an Elmscope I on Gevaert-Scientia 23D50 plates.

RESULTS

The electron microscopic identification of a virus using old diseased samples from the skins of infected chamois is not possible with our centrifuge technique. The shadowing prepared with the latest modifications was son abundant on the non-specific material, in spite of washing, that it could scarcely be seen through. Moreover, the quantity of virus in the old samples appeared to be too small to be detected under the electron microscope. Subsequently, additional investigations on this material were given up and virus identification was carried out only on materials from freshly infected experimental animals (chamois, sheep, and goats). In the papulo-vesiculosum stage, it was possible to observe a virus which corresponded morphologically (size, shape, ultra-structure, and axial ratio) with the E.C. virus of sheep and goats. The fourth day of illness postinfection was found to be the most favorable for detection of the virus. In the crusty lesions which appeared by the sixth day postinfection, virus could no longer be detected by the indirect spot test of Peters (23) since the abundant nonspecific material hindered

detection. After the introduction of a centrifugation program, it was possible, however, to concentrate the virus in the scaby material and to prepare somewhat pure preparations. After 14 days, old crusty lesions, as well as papillomatotic lesions can rarely be used for virus detection by means of the electron microscope. In this case, one must transfer the infectious material to suitable experimental animals or grow the virus in a suitable tissue culture. After the use of either of these procedures, the electron microscopic detection of viruses can be carried out without difficulty.

In one case, we were successful in detecting the E.C. virus with the indirect spot method for papulo-vesiculosum lesions on the upper lip of a young chamois. The oldest of these lesions was estimated to be about four days old.

The skin lesions during the early stages had extremely high virus contents in wild animals as well as in materials obtained from experimental animals. The viruses lay imbedded for the most part in reticular material orginating from the degeneration of cells. They are arranged in singles, pairs, groupe, chains, and in plaques in great numbers (Figures 1, 2, 3, 5, and 6). By means of uranyl acetate negative staining, it was quite easy to differentiate the viruses from the nonspecific material of the same size range. The greatest part of them had the shape of anrotational ellipsoid (9, 10) whose periphery is distinctly compressed (Figures 1 and 2). At higher electron microscope magnifications, a distinct envelope (Fig. 2) can be seen. On the surface, a striated pattern is arranged, which, in many cases, assumes a cross and transverse pattern (figures 2 and 3). This pattern, which is mainly on the surface of the virus, is caused by parallel-arranged filamentous compressions which rung in a diagonal direction on the virus. These cross and transverse markings are probably projections of the reverse side through to the front side. In several preparations, isolated round virus forms (Figure 4) were observed in which case it was difficult to

differentiate the filaments. In the photographs with higher resolution, measurements were made of the distance between the parallel filaments which amounted to 116 Å to 125 Å. The width of the filaments was measured to be 60 Å. Within a single filament, an axial structure of about 20 Å was observed. In the interior of the virus, one can observe thickened area which exhibit an average size of ca. 90 mμ by 65 mμ and lay partly in the center and partly subpolar (Fig. 3). As to the question of this internal body being analogous to that of the Vaccinia virus, one can only speculate at this time. When 100 viruses were measured, the following values were obtained: the long axis varies between 200 mμ and 325 mμ with a mean of 259.75 mμ; the short axis from 150 mμ to 185 mμ with a mean of 154.62 mμ. The axial ratios lay between 1.285 and 2.333 averaging 1.66. A morphological distinction between the infectious agent of contagious pustular dermatitis of chamois (early papillomatosis) and the virus of contagious pustular dermatitis of sheep and goats could not be made. Consequently, through experimental investigations (6) one can confirm the identity of the virus of "papillomatosis" of chamois to be the same as that of contagious pustular dermititis of sheep and goats.

DISCUSSION

The method described by Peters and co-workers (24) for the rapid, electron microscopic diagnosis of variola virus (fixation: osmium tetroxide and negative staining: uranyl acetate) were also applied in the present investigations for the detection of the E.C. virus of chamois and experimental animals. With the uranyl salt, one could find structures similar to these first seen by Nagington and Horne (18) later by Buttner, Giese, Muller, and Peters (3) using phosphotungstic acid (PTA).

The introduction of PTA negative staining by Brenner and Horne (2) in the electron microscopic preparative technique led to the disclosure of sub-structures in viruses which had not previously been seen with the methods employed (osmium fixation and shadowing). A explanation for the negative staining was given by Muller and Meyerhoff (16) later. According to them, in the case of PTA negative staining, there is an electron charge of the micro-region caused by movement of electrons. Moreover, there develops an electrostatic lens through which the electron beam is focused in the micro-region. Muller and Giese (15) believe that in the case of uranyl acetate negative staining, similar physical conditions are displayed. In repeating the studies of Muller and Meyerhoff (16) with uranyl acetate, we observed that latex balls negatively stained with it appear transparent under the electron microscope when they are not treated. If a carbon evaporation coating is employed as a carrier film, then the anomalous contrast (16) was no longer observed, since the electrostatic charge is terminated by the conducting carbon film. In Figure 6, gross charge phenomena are recognized after treatment of a virus sample by uranyl acetate negative staining. Such charge phenomena are thus always observed when a large number of viruses (a virus cluster) is used and a large quantity of uranyl acetate is deposited. In the case of the Vaccinia virus, the substrate was not preserved by uranyl acetate negative staining as it has been described by other authors (21, 22, 7). In the case of this virus, the exterior protein layer probably prohibits the penetration of uranyl acetate (with its pH of 4.2) in sufficient quantities. In the case of the E.C. virus, the different virus types observed with PTA negative staining could not be found since the pH of the uranyl salt could not be shifted towards neutrality with precipitation. The values given in the literature (1, 18, 19, 3) for the size and axial ratio of the E.C. virus are in aggreement with our investigations. The discrepencies are probably the

result of the different preparative techniques employed by the various authors. In table 1 is a summary of the mean values given in the literature for the long and short axes and the axial ratios. The values are express in mμ.

TABLE 1

Investigator	Length	Mean	Width	Mean	Axial ratio L:W
Abdussalam (1)	185-310	251.8	120-210	158.18	1.6
Nagington (17,18)	220-300	273.1	150-175	170.40	1.5
Knocke (11)	220-250	?	ca. 150	?	?
Buttner (3)	?	300.0	?	170.0	1.7
Gerstl (chamois)	200-325	259.75	150-185	154.62	1.68

The clinical diagnosis of contagious pustular dermatitis of sheep on the basis of the characteristic clinical picture appears to pose no particular difficulty, since in an animal stock, several animals with symptoms of different stages of the disease are certainly to be found. In the case of wild animals, on the other hand, the diagnosis, if necessary, can be made only with difficulty. As the virus identification, as mentioned, can be carried out successfully under the electron microscope only in early stages of the disease, one must employ infectious materials from experimental animals or grow the virus in tissue cultures when dealing with old cases of the disease. The subsequent identification of the virus under the electron microscope is then quite easy.

SUMMARY

In the paper presented, the author has reported on the electron microscopic determination of the virus producing "papillomatosis" in the chamois. No

morphologic difference was detectable between this agent and the infectious agent producing contagious pustular dermatitis in sheep and goats. The identity of both agents, already presumed on the basis of experimental results, was thus confirmed electron microscopically. The Ecthyma contagiosum virus was prepared employing a saturated solution of uranyl acetate. By means of this method, the surface structure of the virus was visualized. The physical prerequisites (negative staining) ~~have been~~ discussed.

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FIGURES

The Xerox copy of the paper furnished to the Translator was of such poor quality that the figures contained in it could not be reproduced for use in this translation. As a result, the reader is referred to the original journal for the electron micrographs.

Figure 1. Ecthyma contagiosum virus treated with uranyl acetate. Magnified 20,000 X.

Figure 2. Representation of ultra-structure. R- round elementary bodies; H - envelope membrane. Magnified 80,000 X.

Figure 3. E.C. virus with concentrated area within. Magnified 80,000 X.

Figure 4. Round E.C. Virus. Substructure preserved poorly. Magnified 160,000 X.

Figure 5. Rotational ellipsoidal elementary body shadowed with gold-palladium. Magnified 20,000 X.

Figure 6. Virus cluster, strong charge phenomenon at micro-region. Magnified 40,000 X.